U.S. ARMY MEDICAL RESEARCH
INSTITUTE OF CHEMICAL DEFENSE

USAMRICD-TR-92-01

ESTIMATE OF THE LOWEST DOSE OF DIAZEPAM REQUIRED TO TREAT SOMAN-INDUCED CONVULSIONS IN RHESUS MONKEYS PRETREATED WITH PYRIDOSTIGMINE AND TREATED WITH ATROPINE, PRALIDOXIME CHLORIDE AND DIAZEPAM

Jurgen D. von Bredow
Nancy K. Jaax
Isaac J. Hayward
John V. Wade
Georgia F. Maitland
Andris Kaminski

April 1992

Approved for public release; distribution unlimited

U.S. ARMY MEDICAL RESEARCH INSTITUTE OF CHEMICAL DEFENSE
Aberdeen Proving Ground, MD 21010-5425

DISPOSITION INSTRUCTIONS

Destroy this report when no longer needed. Do not return to the originator.

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.

In conducting the work described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals" as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council.

The use of trade names does not constitute an official endorsement or approval of the use of such commercial hardware or software. This document may not be cited for purposes of advertisement.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	April 1992	Final Jan 88 to Dec 88

4. TITLE AND SUBTITLE
(U)Estimate of the Lowest Dose of Diazepam Required to Treat Soman-induced Convulsions in Rhesus Monkeys Pretreated with Pyridostigmine and Treated with Atropine, Pralidoxime Chloride and Diazepam

5. FUNDING NUMBERS
62787A
3M162787A875 AA

6. AUTHOR(S)
von Bredow, JD, Jaax, NK, Hayward, IJ, Wade, JV, Maitland, GF, and Kaminskis, A

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research Institute of Chemical Defense
ATTN: SGRD-UV-P
Aberdeen Proving Ground, MD 21010-5425

8. PERFORMING ORGANIZATION REPORT NUMBER
USAMRICD-TR-92-01

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research Institute of Chemical Defense
ATTN: SGRD-UV-RC
Aberdeen Proving Ground, MD 21010-5425

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

The purpose of this study was to estimate the minimal dose of intramuscularly administered diazepam to limit soman-induced convulsions in rhesus monkeys pretreated with pyridostigmine and treated with atropine and 2-PAM. Eleven rhesus monkeys were pretreated with four oral doses of pyridostigmine administered at eight-hour intervals before intramuscular administration of 5 LD50 of soman. All of the animals were treated with atropine and 2-PAM chloride one minute following exposure to soman. Nine of the eleven animals were also treated with varying intramuscular doses of diazepam immediately following the injection of PAM-Cl. The clinical course of recovery of soman intoxication was monitored to identify the presence or absence of tonic-clonic convulsive movements. Two soman-exposed animals that received no diazepam convulsed severely and ultimately lapsed into a prolonged coma. In the two animals treated with 50 ug/kg diazepam, convulsions were prevented in one animal but not the other. When four animals were exposed to soman and treated with atropine, 2-PAM and 100 ug/kg of diazepam, brief convulsions occurred in only one of the four

14. SUBJECT TERMS
diazepam, rhesus monkeys, intramuscular, anticonvulsant, laboratory animal

15. NUMBER OF PAGES
44

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	unlimited

Block 13 cont'd.

UNCLASSIFIED

animals. A dose of either 150 or 250 ug/kg of diazepam prevented soman induced convulsions in each of the animals tested at this dose. This study confirmed the ability of diazepam to decrease the severity of convulsive movements in pyridostigmine pretreated primates which had been intoxicated with 5 LD50 of soman and treated with atropine, 2-PAM and various doses of diazepam. Within the limitations of the numbers of animals employed, 100 ug/kg is a minimal dose of diazepam which will prevent tonic-clonic convulsive movements in most pyridsotigmine pretreated, soman exposed, atropine, 2-PAM and diazepam treated rhesus monkeys.

Preface

The work described in this report wi authorized under USAMRICD protocol 1-21-87-000-A-469, entitled "Estimate of the Lowest Dose of Diazepam Required to Trate Anticholinesterase Agent-induced Convulsions in Pyridostigmine-pretreated, atropine, 2-PAM and Diazepam Treated Rhesus Monkeys." Work was started in April 1988 and completed in December 1988. The experimental data are recorded in notebook 047-88.

This work has been previously reported, in part, in the Proceedings of the 1989 Medical Defense Bioscience Review. See Solana, R.P., Corcoran, K.D., von Bredow, J.D., and Lukey, B.J., "Efficacy for Inhibition of Soman-induced Convulsions: Interspecies Scaling from Monkey to Man," pp 459-462, 1989. AD B139550.

Acknowledgements

We express our appreciation for the superb technical support of SSG Dennis Davis, SGT Brian Ling, and Sp4 Scott Heykamp.

Table of Contents

Introduction

In the clinical course of anticholinesterase agent intoxication, non-human primates exhibit an acute onset of ataxia, fasciculations tremors and collapse, followed by unconsciousness, respiratory halt, and death within ten minutes (1). Unconsciousness is usually accompanied by severe motor convulsions. The severity of the convulsions is often related to the time to death following the onset of acute signs. Animals that do not succumb immediately often have alternating periods of severe convulsions and quiescence. Many animals die without regaining consciousness; however, some survive and appear to recover.

Treatment of soman-intoxicated primates with atropine and 2-PAM leads to only limited improvement in survival. Primates exposed to soman in excess of 2 LD50 succumb in spite of atropine and oxime therapy, although the time to death is often prolonged significantly. This extended period following exposure to soman is marked by severe tonic-clonic convulsions (2).

The pretreatment of primates with the carbamate pyridostigmine followed by atropine and 2-PAM therapy results in a significant improvement in the ability of primates to survive exposure to multi-lethal concentrations of soman (2,3,4). The initial pattern of soman intoxication in pyridostigmine pretreated primates is similar to unpretreated control animals in that the animals still suffer a rapid loss of consciousness, followed by the onset of severe convulsions which may persist for thirty minutes to two hours before the animal begins a slow recovery of consciousness. Within 24 to 72 hours, the animals appear to recover; upon autopsy, however, it is apparent that some areas of the central nervous system are severely and potentially irreversibly affected (5,6). This pathology has been shown to be related to the severity of the seizures and convulsive activity observed following exposure to soman (7,8,9,10,11,12).

In soman-intoxicated animals that have been treated with atropine, the addition of diazepam either prevents the onset of convulsions or stops the progression of convulsions to the tonic-clonic stage (13,14). Prevention of this progression of convulsive activity, by augmenting atropine and 2-PAM therapy with the intramuscular administration of diazepam, is accompanied by a significant reduction in brain pathology (15,16).

The dose of diazepam utilized successfully in rhesus monkeys by Lipp ranged from 2 mg/kg to 5 mg/kg (17). A recent investigation utilized a dose of 1 mg/kg of diazepam to completely abolish convulsive activity in pyridostigmine pretreated animals that were also treated with atropine and 2-PAM (16). Previous studies demonstrated the effectiveness of 0.1 mg/kg or 1.0 mg/kg midazolam in pyridostigmine pretreated, atropine and 2-PAM treated primates that were exposed to 5 LD50 of soman (20). Other studies in unpretreated animals indicated that the dose of diazepam could be reduced significantly in the presence of 2-PAM and atropine therapy. The lowest estimate of an

effective dose was 0.335 mg/kg of diazepam when administered in combination with an oxime, TMB4, and sufficient atropine therapy (18).

To determine whether a rational dose of diazepam can be added to atropine and 2-PAM therapy, it is important to establish an estimate of the minimal dose of diazepam required to control soman-induced convulsions. In the current study, a limited number of rhesus monkeys were pretreated with pyridostigmine, then intoxicated with soman, and treated with atropine and 2-PAM therapy in a manner similar to a previous study (2). In addition these primates were treated with varying doses of diazepam required to stop the progression of tonic-clonic convulsions.

Materials and Methods

A. Experimental Design

The dose of diazepam that would reduce the progression of convulsive seizures was determined in a minimal number of animals needed to provide a reasonable estimate of the required dose. The experimental design started with an initial dose of diazepam of 0.250 mg/kg which could be increased or decreased by multiples of 0.050 mg/kg in an established pattern depending upon the response of the pyridostigmine pretreated, atropine, 2-PAM and diazepam treated primate following the exposure of the animal to 5 LD50 of soman. The dose of diazepam was reduced by multiples of 0.050 mg/kg in succeeding animals until a dose of diazepam was established that would not counteract the soman-induced convulsive episode. The dose of diazepam was then increased to the lowest effective dose that was capable of suppressing convulsions. The entire experimental design is illustrated in Table 1.

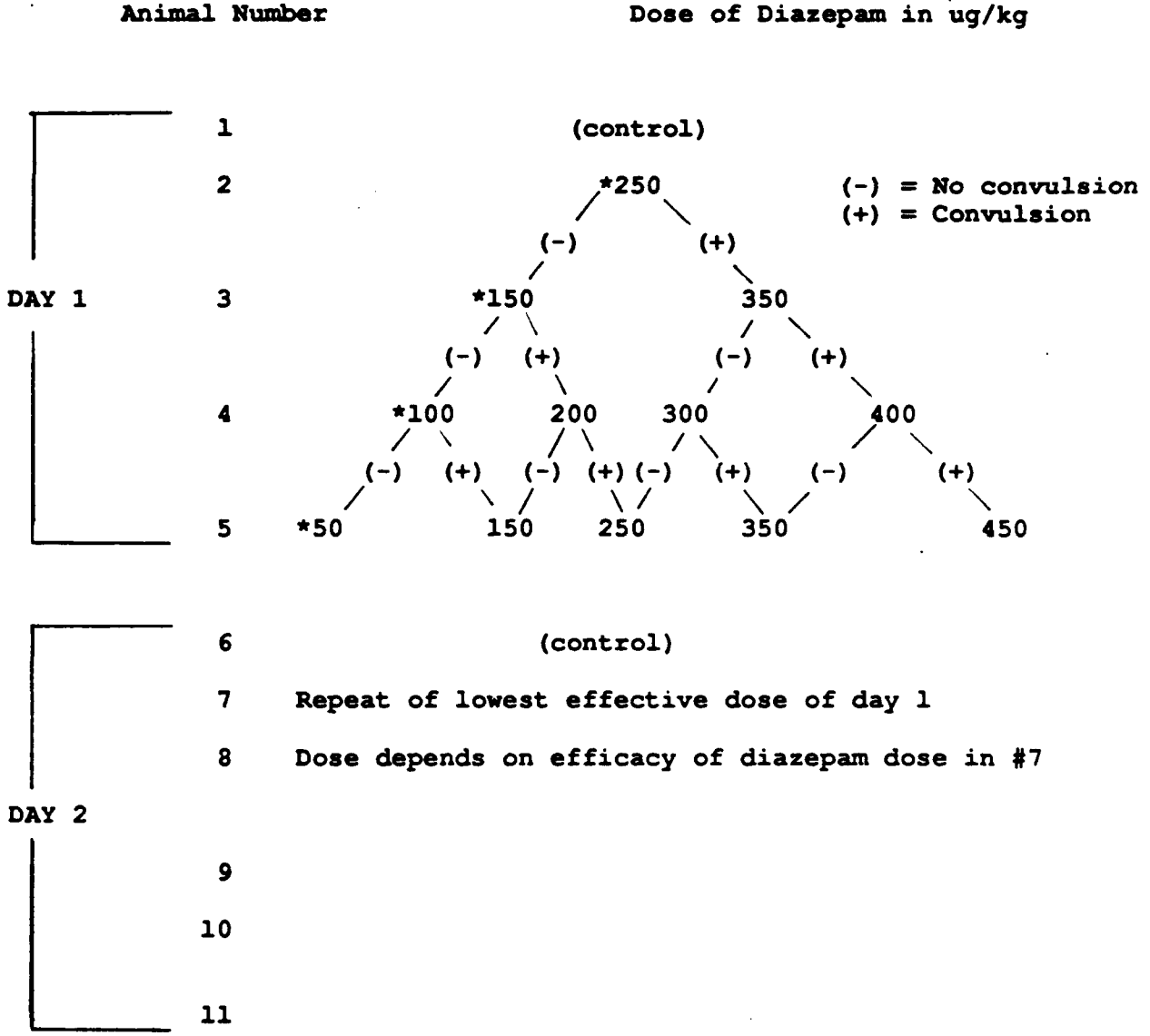
B. Materials

(1) Pyridostigmine bromide, USP (Mestinon Syrup) (Hoffman-LaRoche Inc., Nutley, N.J.). Four 1.2 mg/kg oral doses of Mestinon Syrup (12 mg pyridostigmine bromide/ml) were administered at eight-hour intervals by nasogastric tube (infant feeding tube) passed into the stomach of the animal.

(2) Soman (Pinacolyl methylphosphonofluoridate, GD), USAMRICD 2 mg/ml in saline; additional dilutions were made in saline. A mouse agent (soman) potency test was carried out within one hour before exposure of primates. Five LD50 of soman, defined as 76.5 ug/kg, were injected into the gastrocnemius muscle mass with a 1 cc glass syringe equipped with a 25 gauge 5/8 inch hypodermic needle. The remaining soman was destroyed by dissolution into concentrated sodium hydroxide in accordance with USAMRICD SOP SGRD-UV-PB-1-83.

Table #1

Scheduled Use of Each Animal



* denotes doses of diazepam actually used

(3) Atropine, USP (WRAIR Div of Experimental Therapeutics), 0.4 mg/kg was administered intramuscularly in the quadriceps muscle mass one minute following exposure to 5 LD50 soman using a disposable 25 gauge 5/8 inch hypodermic needle attached to a 1.0 ml glass syringe.

(4) Pralidoxime Chloride (2-PAM) (WRAIR Div of Experimental Therapeutics) 25.7 mg/kg was administered intramuscularly into the quadriceps muscle mass with a 1.0 ml glass syringe attached to a 25 gauge 5/8 inch hypodermic needle immediately following the injection of atropine and near the same atropine injection site.

(5) Diazepam 5 mg/ml (Elkins-Sinn Inc., Cherry Hill, N.J.). The dilutions of diazepam were prepared in the FDA approved vehicle consisting of 40% propylene glycol, 10% ethanol and 50% water. All injection volumes of diazepam were developed in a manner that maintained a constant injection volume of 0.1 ml/kg regardless of the dose of diazepam. The dose of diazepam or the diazepam vehicle was injected intramuscularly into the quadriceps muscle mass immediately following the injection of 2-PAM and near the same oxime injection site.

(6) A total of eleven male rhesus monkeys (2.9 to 5.5 kg) were supplied by the Veterinary Medicine and Surgery Branch.

C. Methods

The animals were pretreated with four oral doses of 1.2 mg/kg pyridostigmine administered at eight-hour intervals. Four hours and ten minutes following the last dose of pyridostigmine, a blood sample was withdrawn to determine the red cell cholinesterase activity. Red cell cholinesterase activity was determined by a modification of the automated delta pH technique (22). The mean pyridostigmine-induced inhibition of red cell cholinesterase activity was 36% before exposure to soman. The red cell cholinesterase inhibition of each animal is indicated in the detailed clinical description in the appendix to this report. Four and one-half hours following the last oral dose of pyridostigmine, the animals were exposed intramuscularly to 5 LD50 (76.5 ug/kg) of soman and treated one minute later with 0.4 mg/kg atropine sulfate, 25.7 mg/kg 2-PAM chloride and varying doses of diazepam. Each of these therapeutic doses was administered separately at a different quadriceps muscle (vastus lateralis) site of the right hind limb of the animal. The animals were restrained manually during the dosing procedures and throughout the initial observation period. The time of the onset and the progression of severe signs of soman intoxication were carefully monitored to establish the anticonvulsant effect of varying doses of diazepam. The anticonvulsant efficacy of varying doses of diazepam is defined in Table 2. The animals were returned to their holding cages as soon as convulsions had ceased and vital signs were stable.

Results

The minimal dose of diazepam required to limit soman-induced convulsions in pyridostigmine pretreated, atropine and 2-PAM treated primates is shown in Table 2.

As indicated in Table 2, the combination of pyridostigmine pretreatment followed by atropine and 2-PAM therapy without diazepam caused severe convulsions in two control animals. The lowest effective dose of diazepam (50 ug/kg) reduced convulsions in one of two subjects. The dose of 100 ug/kg was effective in three of four animals. Lack of convulsions at 150 ug/kg and 250 ug/kg in only one animal at each dose suggests that a dose greater than 100 ug/kg may prevent convulsions in all animals. Based on these observations, 100 ug/kg was accepted as a minimal dose of diazepam that will prevent convulsions in most animals.

Table 2

Dose of Diazepam ug/kg i.m.	# of Animals	Observation	Mortality
0	2	Both animals convulsed	One died at 72 hrs
50	2	1/2 no convulsions	
100	4(5)	3/4 no convulsions	Fifth died at 9 mins
150	1	No convulsions	
250	1	No convulsions	Died at 40 hrs

The entire clinical course of soman intoxication and recovery of each animal is described in the attached appendix.

Discussion

This preliminary study confirmed the ability of lower doses of diazepam than had previously been reported to decrease the severity of convulsive movement in carbamate pretreated primates that had been intoxicated with soman and then treated with atropine, oxime and various doses of diazepam. Reduction in observed convulsive movements does not necessarily guarantee a complete abolition of electrical seizure

activity of the central nervous system. Although the animals become quiescent and may even appear to be in a coma-like state, the central nervous system may continue to endure severe seizure activity at various sites. This continued seizure activity may still be associated with the development of lesions in the CNS (23). The ability to determine whether a minimal dose of diazepam will ensure a halt of the electrical seizure activity in the central nervous system pathology was not within the scope of this study.

An effort was made to carry out this study in a manner similar to a pyridostigmine efficacy study completed previously (2). The method used in that study attempted to simulate field conditions by pretreating the animals with six oral doses of pyridostigmine before exposure to a severe challenge with soman, followed one minute later by therapy with atropine and 2-PAM chloride. In this study, only four oral doses of pyridostigmine were administered to the primates before exposure to soman, which was sufficient to provide comparable red cell cholinesterase inhibition.

The current study used the same techniques in two control animals and nine experimental animals treated with varying doses of diazepam. As indicated in the clinical observation data sheets (appendix), both of the control animals demonstrated all of the signs of intoxication described in previous reports. Although these animals had been pretreated with pyridostigmine, followed by atropine and 2-PAM therapy, the animals became unconscious and convulsed severely for a period of time, then became quiescent and periodically convulsed again in a severe tonic-clonic pattern.

One of the control animals cycled between severe convulsions and quiescence for a period of nearly three hours. In previous protocols, animals that convulsed severely and remained unconscious for several hours developed a poor prognosis for ultimate survival; therefore, this control animal was treated with a dose of 250 ug/kg of diazepam near the end of the three hour observation period. It is interesting to note that this relatively low dose (compared to doses employed in previous protocols) readily halted the severe convulsions within three minutes. In spite of the previous convulsive cycling episodes, the convulsive movements never returned in this animal, and the animal regained consciousness within two hours following the administration of diazepam. This observation demonstrates that diazepam may be effective not only in limiting the convulsive episode but also in allowing a more rapid return of consciousness in some cases.

This is an important observation since there has been some concern as to whether diazepam could function as an anticonvulsant to stop convulsions once they have proceeded for a period of time. This assumption has been emphasized by a study reported at the 1988 RSG-3 conference (19) which stated that seizure activity could not be halted by diazepam once the convulsive episode, induced by soman in rats, was well under way. Our observation suggests that diazepam may be effective even if given some time late in the course of soman intoxication.

The 5 LD50 challenge dose of soman developed as part of a previous study (2) is significantly greater than has been reported in other literature sources. A two-fold increase in the amount of soman administered represents a very severe challenge (in excess of 10 LD50). Although the research of Dirnhuber (21) indicated that the efficacy of carbamate pretreatment in primates should protect the animals from intoxication by an excess of 25 LD50 soman, the potential of encountering non-survivors increases with extreme challenge. Therefore, although some animals can recover following an exceedingly high dose of agent, this may not be the case for all animals. Utilization of the high LD50 value of soman [15.3 ug/kg (2) instead of the reported 6.6 ug/kg (1)] may have contributed to the unexpected death of animal #2, #6 and #11. Attempting to develop a treatment regimen that is capable of protecting all animals at a realistic exposure to soman (5 LD50) is more important than achieving a system that will protect some animals against exceedingly high doses of soman.

Delayed death or failure of a pyridostigmine pretreated, soman-exposed, atropine, 2-PAM and diazepam treated animal to regain consciousness was unexpected, but has been reported before (2). Not all carbamate pretreated animals that have been exposed to high doses of soman and treated with atropine and 2-PAM can be expected to survive; nevertheless the survival rate is at least 90%.

The data provided by animal #2, which expired on the second day of the study, is still useful. The ability to control convulsions during the initial phase was clearly evident. The dose of diazepam (250 ug/kg) is probably not responsible for the cause of death in this animal since the control animal #1, treated under identical circumstances except that the diazepam was administered nearly three hours after exposure to soman, responded exceptionally well to the anticonvulsant. If diazepam were to be considered responsible for the lack of recovery of consciousness and ultimate demise of animal #2, certainly control animal #1 should have also succumbed. Nevertheless, this animal demonstrated an apparently complete recovery following the administration of the same 250 ug/kg dose of diazepam.

The prolonged apnea and very sudden death of animal #11 is not the usual clinical course in carbamate pretreated, antidote treated animals. Since the animal died before the usual onset of soman-induced convulsions, animal #11 was eliminated from this study. Sudden death following a halt in respiration is the most common characteristic of animals which have neither been pretreated nor treated following exposure to soman. The second control animal (animal #6) receiving no diazepam, but being pretreated with pyridostigmine and therapy with atropine and 2-PAM, developed the classical signs of severe convulsions and loss of consciousness from which the animal slowly recovered. The apparent complete recovery of this animal, to the extent that the animal was moving about normally in the cage without difficulty, prior to sudden, unexpected death, has not been observed before. Since the recovery appeared normal, no additional precautions or observation times were scheduled and the animal expired unobserved.

The potential for neuropathology is expected in the two control animals, which were not treated with diazepam, and potentially in the two animals that convulsed in spite of low dose diazepam therapy. Animals that failed to produce tonic-clonic convulsions may have been spared from neuropathology; however, validation of this aspect of diazepam therapy is beyond the scope of this study. The scope of this preliminary study is also limited by the minimal number of animals utilized the experimental design, Table #1. The goal of this study was directed toward determining whether a dose of diazepam lower than had been used in previous studies would prevent the onset of tonic-clonic convulsions. Since a lower dose of diazepam prevented convulsions and attempt was made to estimate the required dose within 50 ug/kg. Due to the restricted number of primates and the non-geometric design of the diazepam dose groups, the standard techniques of median dose determination could not be applied (24).

Conclusion

In pyridostigmine pretreated, soman exposed, atropine, 2-PAM and diazepam treated rhesus monkeys, 100 ug/kg approximates the minimal dose of diazepam that will prevent convulsions in most animals.

References

1. Adams, N.L., von Bredow, J.D., and DeVera, H.V. Intramuscular Lethality of GD (Soman) in the Rhesus Monkey (U). Edgewood Arsenal Technical Report EB-TR-76039 (1976). AD A026821

2. Kluwe, W. M., Chinn, J.C., Feder,P., Olson, C. and Joiner, R., Efficacy of Pyridostigmine Pretreatment Against Acute Soman Intoxication in a Primate Model. Proceedings of the Sixth Medical Chemical Defense Bioscience Review, 227-234, 4-6 August 1987.
AD B121516

3. McGarrigle, R.E., Adams, N.L., von Bredow, J.D., and Steinberg, G.M. The Effectiveness of a Carbamate in Prophylaxis Against Nerve Agent in Dogs and Monkeys (U). Edgewood Arsenal Technical Report EB-TR 76034 (1976).

4. von Bredow, J.D., McGarrigle, R.E., Adams, N.L. and Vick, J.A. Carbamate and Anticholinergic Prophylaxis Against Multilethal Concentrations of Soman in Primates. The Pharmacologist 24: 220 (1982).

5. Wall, H.G., Jaax, N.K., and Hayward, I.J. Brain Lesions in Rhesus Monkeys After Acute Soman Intoxication. Proceedings of the Sixth Medical Chemical Defense Bioscience Review, 155-162, 4-6 August 1987. AD B121516

6. Petras, J.M. Brain Pathology Induced by Organophosphate Poisoning with the Nerve Agent Soman. Proceedings of the Fourth Annual Chemical Defense Bioscience Review, 407-414, 30 May - 1 June 1984. AD B089975

7. McDonough, J.H., Nipwoda, M., Smith, M. and McLeod, C.G. Production of Seizures and Seizure-Related Brain Damage by Direct Microinjections of Nerve Agent into the Brain. Proceedings of the Fifth Annual Chemical Defense Bioscience Review, 439-453, 29-31 May 1985. AD B104126

8. Wall, H.G., McLeod, C.G., Hutchinson, L.S. and Shutz, M. Brain Lesions in Rats Surviving Soman Induced Convulsions: Light and Electron Microscopy. Proceedings of the Fifth Annual Chemical Defense Bioscience Review, 457-473, 29-31 May 1985. AD B104126

9. Adams, N.L., Koviak, T., Hallowell, M., Moffitt, J.T. and Jaax, N.K. The Relationship of Soman-Induced Seizures to Brain Pathology. Proceedings of the Sixth Medical Chemical Defense Bioscience Review, 451-454, 4-6 Aug 1987. AD B121516

10. Lemercier, G., Carpentier, Sentenac-Roumanou, H., and Morelis, P. Histological and Histochemical Changes in the Central Nervous System of the Rat Poisoned by an Irreversible Anticholinesterase Organophosphorus Compound. Acta Neuropathol. (Berl), 61, 123-129, 1983.

11. McLeod, C.G., Singer, A.W., and Harrington, D.G. Acute Neuropathology in Soman Poisoned Rats. Neurotoxicology 5, 53-58, 1984.

12. Petras, J.M. Soman Neurotoxicity. Fund Appl Toxicol 1, 242, 1982.

13. Sarvey, J.M. and Williamson, A.M. Diazepam and Barbiturates Antagonize the Effect of an Organophosphate Anticholinesterase in Rat Hippocampal Slice. Proceedings of the Sixth Medical Chemical Defense Bioscience Review, 421-423, 4-6 Aug 1987. AD B121516

14. Shih, T. and Koviak, T. Evaluation of the Anticonvulsant Effects of Diazepam and MK-801 in Soman Poisoning. Proceedings NATO Research Study Group Panel VIII/RSG-3, 179-202, 26-29 September 1988.

15. Churchill, L., Pazdernik, T.L., Nelson, S.R. and Samson, F. E. Muscarinic Receptor Maps Reveal Selective Brain Damage in Soman Exposed Rats; Diazepam Prevents this Damage. Proceedings of the Fifth Annual Chemical Defense Bioscience Review, 475-488, 29-31 May 1985. AD B104126

16. Hayward, I.J., Wall, H.G., Jaax, N.K., Wade, J.V., Nold, J.B. and Marlow, D.D. Influence of Therapy with Anticonvulsant Compounds on the Effects of Acute Soman Intoxication in Rhesus Monkeys. Proceedings NATO Research Study Group Panel VIII/RSG-3, 141-177, 26-29 September 1988.

17. Lipp, J.A. Effect of Diazepam Upon Soman-Induced Seizure Activity and Convulsions. Electroenceph. Clin. Neurophysiol., 32, 557-560, 1972.

18. Adams, N.L. Prophylaxis and Therapy Against Soman Poisoning in the Cynomolgus Monkey. Technical Report, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD (in preparation).

19. Riotte, M., Vacquier, M. and Blanchet, G. Efficacite de queiques benzodiazepines administrees a titre curatif chez le singe et le cobaye intoxiques par le soman. NATO Research Group Panel VIII/RSG-3, 203-217, 26-29 September 1988.

20. von Bredow, J., Maitland, G., Pate, C., Kaminskis, A., Adams, N., Wall, H. and Jaax, N., Determination of the Effectiveness of Midazolam in Preventing Soman-induced Convulsions and Neuropathology in Macaca Fascicularis. USAMRICD-TR-90-14, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD, December 1990. AD A232017

21. Dirnhuber, P., French, M.C., Green, D.M. Leadbeater, L. and Stratton, J.A. The Protection of Primates Against Soman Poisoning by Pretreatment with Pyridostigmine. J. Pharm. Pharmacol., 31, 295-299, 1979.

22. Groff, W.A., Kaminskis, A. and Ellin, R.I. Interconversion of Cholinesterase Enzyme Activity Units by the Manual Delta pH Method and a Recommended Automated Method, Clin. Tox., 9(3), 353-358, 1976.

23. Meldrum, B.S., Vigouroux, R.A. and Brierley, J.B. Systemic Factors and Epileptic Brain Damage, Arch. Neurol., 29, 82-87, 1973.

24. Weil, C.S. Tables for Convenient Calculation of Median Effective Dose (LD50 or ED50) and Instructions in Their Use. Biometrics, 8(3), 249-263, 1952.

Appendix

Clinical Course of Intoxication and Recovery Following Intoxication by Soman

MONKEY #1

Time	Event
-4 hr, 30 min	Monkey #143, weighing 4.6 kg, was provided with the last oral dose of mestinon equivalent to 1.2 mg/kg of pyridostigmine.
-20 min	2 ml blood sample was drawn for cholinesterase activity determination. Red cell acetylcholinesterase activity was inhibited 43%.
0 min	5 LD50 (76.5 ug/kg) of soman was administered into the gastrocnemius muscle mass of the left hind limb.
1 min	Atropine, 2-PAM and diazepam vehicle were administered intramuscularly into the quadriceps muscle mass of the right hind limb.
1 min, 40 sec	The onset of signs of intoxication occurred with some severe tremors along the back and all four limbs of the animal.
2 min, 30 sec	The animal was lying unconscious on its side, with its eyelids blinking rapidly.
3 min, 30 sec	Prolonged apnea, followed by several inspiratory gasps.
5 min	The animal continued to lie unconscious on its side; a periodic trembling of all four limbs was evident.
8 min	The animal continued to lie on its side exhibiting severe tremors and some salivation in spite of the atropine therapy.
9 min	The severe tremors progressed into signs of convulsions with the tail lashing wildly about.
12 min	The animal continued to lie unconscious on its side demonstrating some mild convulsive movements.
22 min	The animal remained unconscious lying on its side with periodic jerky movements of the limbs.
32 min	The animal developed severe tonic-clonic convulsions with tail lashing and opisthotonos.
34 min	The animal became quieter and the convulsions ceased.

40 min	Periodically there were severe tremors which subsided and the animal returned to an unresponsive coma.
1 hr	The animal was still unconscious; the vital signs were stable and the animal was returned to its cage.
1 hr, 6 min	The animal suddenly became conscious and was able to sit up.
1 hr, 40 min	The animal was able to stand up and to remain standing by holding on to the side of the cage.
1 hr, 50 min	The animal became hyper-reactive to sound and visual stimuli.
2 hr, 4 min	The animal lapsed into a second period of unconsciousness and severe tonic-clonic convulsions.
2 hr, 13 min	The convulsions subsided momentarily, but the animal remained unconscious.
2 hr, 14 min	The convulsive movements were enhanced.
2 hr, 20 min	The convulsions stopped momentarily and then increased again.
2 hr, 30 min	400 ug/kg of atropine was injected intramuscularly into the quadriceps of the left hind limb. There was no immediate effect on the convulsions or state of consciousness.
2 hr, 40 min	The severe convulsions continued without any signs of improvement.
2 hr, 45 min	The severe convulsive movements continued; the heart rate was 180 BPM. Respiration was mildly obstructed in spite of the atropine therapy.
2 hr, 48 min	250 ug/kg of diazepam were injected intramuscularly into the quadriceps of the right hind limb.
2 hr, 51 min	The convulsions stopped suddenly. The animal remained lying on the floor of the cage completely unconscious. Heart rate increased to 220 BPM, respiration remained deep, clear and slower than normal. Although the convulsions stopped, the animal remained completely unconscious, unresponsive to touch or light and developed some uncoordinated chewing movements.
4 hr	The animal remained lying on the floor of the cage unconscious, unresponsive to touch, with some fasciculations along the back and legs.

6 hr	The animal was sitting up quietly with its head in a downward position. Some minor fasciculations continued.
8 hr	The animal was lying quietly on the floor of the cage. Animal was alert and accepted half of an orange piece and began eating.
20 hr	The animal was apparently normal and was able to eat four monkey chow biscuits and 1/4 of an orange.
24 hr	The animal was completely alert, active and eating in an apparently normal manner.
36 hr	Animal continued to be completely normal.
48 hr	The animal was normal and did not demonstrate any signs of relapse.

Summary: This is control animal #1 which was initially treated only with Valium vehicle. The animal received no diazepam initially. The animal suffered tonic-clonic convulsions and a loss of consciousness. At 2 hours and 45 minutes it appeared as though the animal would continue to convulse, remain unconscious and not survive. The animal was treated with 250 ug/kg diazepam (the same dose as diazepam used in monkey #2) and the tonic-clonic convulsions subsided within three minutes.

MONKEY #2

TIME	EVENT
-4 hr, 25 min	Monkey #DA 104, weighing 5.5 kg, was given the last oral dose of mestinon equivalent to 1.2 mg/kg pyridostigmine.
-20 min	2 ml blood sample was removed for cholinesterase activity assay. Red cell cholinesterase activity was inhibited 18%.
0 min	5 LD50 (76.5 ug/kg) of soman was injected intra-muscularly into the gastrocnemius muscle mass of the left hind limb.
1 min	Atropine, 2-PAM and 250 ug/kg diazepam were injected intramuscularly into the quadriceps muscle mass of the right hind limb.
2 min, 20 sec	The animal was unconscious, lying on its side with some trembling of the fore limbs.
3 min	The animal was totally unconscious, lying quietly on its side demonstrating no tremors or uncoordinated movements. There was some salivation.
4 min	A momentary respiratory halt was followed by a deep gasp, a period of apnea and, again, a deep gasp. Some tremors of the shoulder area were evident.
5 min	Respiration improved, some fasciculations along the back were evident.
6 min	The animal was unconscious, respiration was adequate and tremors of the shoulder muscles increased, but no convulsions were evident.
9 min	Animal was unconscious, had pin point pupils and did not respond to the light of a flashlight.
13 min	The animal remained quiet and unconscious, the respiration was deep, regular and unobstructed.
15 min	The animal was unconscious, demonstrating no response to light or touch; there was no indication of convulsions.
30 min	The animal continued to be comatose and un-responsive. Vital signs were adequate.

53 min	The animal remained unconscious and unresponsive to light or touch. The animal was returned to its holding cage.
1 hr, 45 min	The animal continued to lie quietly on the floor of the cage. Respiration and vital signs were near normal.
4 hr	Animal continued to be unconscious and unresponsive. No indication of convulsions were present.
8 hr	No significant change.
24 hr	Animal continued to lie quietly on the floor of the cage, unresponsive to light or touch with no indication of convulsions. Vital signs were adequate, and the animal was provided with fluids (Ringers lactate) administered subcutaneously to prevent dehydration.
36 hr	No improvement or signs of recovery, the animal continued to be supported with fluid therapy.
40 hr	Animal expired without showing any signs of recovery of consciousness.

Summary: The animal received 250 ug/kg of diazepam along with the atropine and 2-PAM one minute following the administration of soman. Although the animal demonstrated some tremors along the back and fore limbs as well as some fasciculations, there was no clear indication that tonic-clonic convulsions occurred. Although the animal was unconscious and may have suffered seizures, there were no outward signs of convulsions following the administration of 250 ug/kg of diazepam.

MONKEY #3

Time	Event
-4 hr, 30 min	Monkey #DA158, weighing 3.8 kg, received its last oral dose of mestinon equivalent to 1.2 mg/kg pyridostigmine.
-20 min	2 ml blood sample was drawn for cholinesterase activity determination. Red cell cholinesterase activity was inhibited 39%.
0 min	5 LD50 (76.5 ug/kg) of soman was injected intramuscularly into the gastrocnemius muscle mass of the left hind limb.
1 min	Atropine, 2-PAM and 150 ug/kg diazepam were injected intramuscularly into the quadriceps muscle mass of the right hind limb.
2 min, 30 sec	Animal was unconscious and lying on its side.
3 min	The animal demonstrated some apnea followed by deep inspiratory gasps.
4 min	The animal remained lying quietly on its side completely unconscious; respiration was more regular.
7 min	The animal remained unconscious and unresponsive to touch with deep, rapid and clear respiration.
ll min	The animal continued to be unconscious, lying quietly on its side with some tremors of the head and neck, but without demonstrating signs of convulsions.
15 min	The animal continued to be comatose and unresponsive.
20 min	The animal remained quiet, unresponsive with some uncoordinated movements of fore limbs.
43 min	No significant improvement. Animal remained lying on its side with some uncoordinated movement of the fore limbs. Animal was returned to the cage.
1 hr, 10 min	The animal exhibited signs of consciousness and made attempts to stand up in the cage.
1 hr, 40 min	The animal stood up and remained standing by holding onto the sides of the cage.

2 hr	The animal moved around in the cage in an ataxic uncoordinated manner.
4 hr	The animal sat up on the floor of the cage completely conscious; some tremors of the shoulders and fore limbs remained.
8 hr	The animal moved about in the cage in a normal manner.
24 hr	The animal continued to respond in a normal manner.
48 hr	The animal continued to respond in a normal manner without any signs of intoxication.

MONKEY #4

Time	Event
-4 hr, 30 min	Monkey #DA155, weighing 4.9 kg, received its last oral dose of mestinon equivalent to 1.2 mg/kg of pyridostigmine.
-20 min	2 ml blood sample was drawn for cholinesterase activity determination. Red cell cholinesterase activity was inhibited 31%.
0 min	5 LD50 (76.5 ug/kg) of soman was injected intramuscularly into the left gastrocnemius muscle mass.
1 min	Atropine, 2-PAM and 100 ug/kg diazepam were injected intramuscularly into the quadriceps muscle mass of the right hind limb.
2 min, 20 sec	Onset of signs of intoxication occurred with tremors and fasiculations along the back. The animal was unconscious and unresponsive to touch. Respiration appeared clear and adequate.
2 min, 40 sec	Respiration stopped suddenly and started again following a period of prolonged apnea.
3 min, 30 sec	There were several periods of prolonged apnea followed by deep gasps.
6 min	The animal continued to be unconscious and unresponsive to touch and to the light of a flashlight.
20 min	The animal continued to be unconscious, but made some uncoordinated movement of the hind limbs; there were no apparent convulsions.
40 min	No significant improvement. The animal was returned to the cage.
1 hr, 30 min	The animal continued lying on its side, with no signs of convulsions.
2 hr, 10 min	The animal stood up and remained standing by holding onto the side of the cage.
2 hr, 30 min	The animal continued to stand up but was completely disoriented and bit the bars of the cage.
6 hr	The animal continued to hold on to the side of the cage and was not able to eat.

8 hr	The animal sat on the floor of the cage, appeared fatigued and depressed.
24 hr	The animal was more alert, but remained sitting on the floor of the cage, still fatigued and depressed.
36 hr	Some mild fasciculations of the lower back continued. The animal appeared nearly normal.
48 hr	The animal was apparently normal and ate without difficulty.

Summary: Although this animal demonstrated tremors, no obvious signs of tonic-clonic convulsions were apparent following therapy with 100 ug/kg of diazepam.

MONKEY #5

Time	Event
-4 hr, 30 min	Monkey #13943, weighing 3.5 kg, was given last oral dose of mestinon equivalent to 1.2 mg/kg pyridostigmine.
-20 min	2 ml of blood was withdrawn for cholinesterase activity. Red cell cholinesterase activity was inhibited 35%.
0 min	5 LD50 (76.5 ug/kg) of soman was administered intramuscularly into the gastrocnemius muscle mass of the left hind limb.
1 min	Atropine, 2-PAM and 50 ug/kg diazepam were administered intramuscularly into the quadriceps muscle mass of the right hind limb.
2 min, 30 sec	The animal suddenly became unconscious and laid down on its side; it demonstrated some trembling of all four limbs, but no signs of convulsions.
3 min, 30 sec	The animal continued to lie on its side. It was unconscious, unresponsive to touch or the light of a flashlight. Minimal tremors in all four legs continued. Respiration remained stable with no signs of apnea.
4 min	The animal demonstrated some response to touch.
6 min	The animal continued to lie on its side, was unconscious and unresponsive to touch.
9 min	There was no significant improvement, the animal remained unconscious.
18 min	The animal was still in an unconscious coma, the pupils were severely constricted.
25 min	The animal remained quiet and unconscious, respiration was deep and clear. The pupils remained severely constricted. There were no visible indications of convulsions.
30 min	The animal continued to lie on its side in an unconscious, unresponsive coma with no indication of convulsions.
50 min	There was no significant change, the animal remained in an unconscious coma.

55 min	The vital signs were within normal limits, the animal remained unconscious and was returned to the holding cage.
1 hr	The animal remained unconscious without visible convulsions.
2 hr	No significant improvement.
4 hr	The animal continued to lie on the floor of the cage unconscious and unresponsive, demonstrating no apparent convulsions.
6 hr	No significant improvement.
8 hr	No apparent improvement.
12 hr	The animal was conscious and moving about in the cage in an uncoordinated manner.
24 hr	The animal appeared to be completely normal.

Summary: The animal continued normal throughout the remainder of the 48-hour observation period without any indication of relapse. Fifty (50) ug/kg diazepam apparently prevented visible signs of convulsions in this soman-intoxicated animal.

MONKEY #6

Time	Event
-4hr, 30min	Monkey #13472, weighing 2.9 kg, was given the last oral dose of mestinon equivalent to 1.2 mg/kg of pyridostigmine.
-20 min	2 ml blood sample was withdrawn for cholinesterase activity determination. Red cell cholinesterase activity was inhibited 40%.
0 min	5 LD50 (76.5 ug/kg) of soman was injected intramuscularly into the gastrocnemius muscle mass of the left hind limb.
1 min	Atropine, 2-PAM and the diazepam vehicle were injected intramuscularly into the quadriceps muscle mass of the right hind limb.
1 min, 30 sec	The animal laid down on its side. The animal became unconscious and unresponsive to touch; respiration was rapid and shallow.
2 min, 20 sec	Respiration had slowed to 6 breaths per minute.
3 min	Periodic apnea, some uncoordinated thrashing of the hind limbs without definite signs of convulsions.
4 min	The animal still suffered some periods of respiratory apnea followed by deep inspiratory gasps.
6 min	Periods of apnea decreased; the animal remained completely unconscious and unresponsive to touch.
10 min	The respiration improved to a more regular pattern; there was no response to touch or to the light of a flashlight. There were no signs of convulsions.
14 min	Tremors of the head and neck increased; some jerking movements of the limbs were observed.
15 min	Some clonic contractions of the fore limbs occurred with some indication of tonic-clonic jerking movements.
19 min	Severe tonic-clonic movements of the limbs occurred.
20 min	The convulsive movements subsided leading to more disorganized tremors of the limbs.

24 min	The tonic-clonic movements developed into severe periodic convulsive movements which would stop suddenly and then reoccur.
27 min	Another violent period of tonic-cloni convulsions occurred and then stopped.
28 min	The animal was quiet, completely unconscious and unresponsive to touch.
29 min	A short period of severe tonic-clonic convulsions occurred.
35 min	Another period of severe tonic-clonic convulsions occurred which were followed by a period of complete quiescence.
1 hr, 15 min	The animal was completely unconscious and unresponsive: it remained lying on its side with no visible convulsive movements.
l hr, 30 min	Vital signs were adequate and the animal was returned to the holding cage in a comatose state.
2 hr	The animal had not regained consciousness, the animal remained completely quiet and unresponsive.
4 hr	No significant change.
8 hr	The animal was unconscious, lying on its side and not responsive to touch, with signs of severe gastrointestinal distress and diarrhea. No signs of convulsive movements were evident.
12 hr	The animal was still unconscious and unresponsive to touch. Signs of convulsions were not evident.
24 hr	The animal was alert and was moving around in the cage without difficulty. The animal was able to eat but demonstrated a slight degree of depression and fatigue.
36 hr	The animal appeared normal.
48 hr	The animal appeared to have recovered completely, was able to move about its cage with ease and responded well to visual and auditory stimuli. The animal ate monkey chow biscuits and appeared to have recovered from the severe GI distress.

72 hr	The animal expired beyond the normal observation period. The exact cause of death could not be determined. However, the necropsy results indicated neuronal degeneration and necrosis. Death may have resulted from severe neuropathology of the hippocampus and cerebrum. No peripheral cause of death could be identified.

Summary: This control animal which did not receive diazepam therapy endured severe convulsions.

MONKEY #7

Time	Event
-4 hr, 30 min	Monkey #13419, weighing 3.0 kg, was given the last oral dose of mestinon equivalent to 1.2 mg/kg of pyridostigmine.
-20 min	2 ml blood sample was withdrawn for cholinesterase activity determination. Red cell cholinesterase activity was inhibited 43%.
0 min	5 LD50 (76.5 ug/kg) of soman was injected intramuscularly into the gastrocnemius muscle mass of the left hind limb.
1 min	Atropine, 2-PAM and 50 ug/kg diazepam were administered intramuscularly into the quadriceps muscle mass of the right hind limb.
2 min	The animal lay its side completely immobile and unconscious.
2 min, 30 sec	There was a prolonged period of respiratory halt, followed by a deep gasp leading to another period of apnea.
3 min, 20 sec	Respiratory halt lasting more than 20 seconds.
4 min	A respiratory halt was followed by a deep respiratory gasp. The animal was completely unconscious exhibiting no movement of the head or limbs.
5 min	Respiration had improved in depth and rate.
6 min	Some tremor of the shoulder muscle was evident.
7 min, 30 sec	Tonic-clonic convulsions occurred.
8 min, 30 sec	Tremors and convulsions persisted.
10 min, 30 sec	Convulsive movements continued in both the fore and hind limbs.
12 min	The convulsive movements became more severe.
13 min, 30 sec	The convulsive movements stopped suddenly.
15 min	The animal was quiet, unconscious and unresponsive to touch.
18 min	Periodic tonic-clonic movements continued. A slight response to the light of a flashlight was evident.

22 min	The animal was unconscious, but periodic running movements of the fore and hind limbs were evident.
36 min	A slight response to touch was evident, and the animal was returned to the holding cage.
45 min	The animal remained on the cage floor, demonstrated a visual response and some uncontrolled movement of the limbs.
1 hr, 15 min	The animal was standing up in the cage, holding on to the side for support. Severe tremors of shoulder muscles persisted.
1 hr, 40	The animal demonstrated a good visual response as well as an intermittent vocal response.
2 hr, 45 min	The animal was sitting quietly in the cage, depressed and fatigued; it no longer demonstrated tremors or convulsive movements.
4 hr	The animal continued to sit quietly in the cage apparently depressed and fatigued.
6 hr	No significant improvement.
8 hr	The animal continued to sit up in an apparently normal condition, but some fatigue was evident.
12 hr	The animal continued to appear normal; it responded aggressively to the animal handler.
24 hr	The animal moved about its holding cage without difficulty. The animal was eating normally and demonstrated no further signs of intoxication.
48 hr	The animal appeared normal and did not suffer a period of relapse.

Summary: This was the second animal that received 50 ug/kg of diazepam. The convulsive movements were not stopped with this dose of diazepam in this animal. Therefore, the dose in succeeding animals was increased to 100 ug/kg.

MONKEY #8

Time	Event
-4 hr, 30 min	Monkey #12430, weighing 3.4 kg, was given the last oral dose of mestinon equivalent to 1.2 mg/kg of pyridostigmine.
-20 min	2 ml blood sample was withdrawn for cholinesterase activity determination. Red cell cholinesterase activity was inhibited 40%.
0 min	5 LD50 (76.5 ug/kg) of soman was injected intramuscularly into the gastrocnemius muscle mass of the left hind limb.
1 min	Atropine, 2-PAM and 100 ug/kg of diazepam was administered intramuscularly into the quadriceps muscle mass of the right hind limb.
2 min	The animal lay down on its side, apparently unconscious.
3 min	The animal was comatose; respiration had ceased completely.
3 min, 30 sec	The animal made a deep respiratory gasp, leading to another period of apnea.
4 min	The animal provided another inspiratory gasp.
5 min	Periods of apnea continued, mixed with respiratory gasps; the animal was totally unconscious and unresponsive to stimuli.
7 min	The depressed respiration and the prolonged periods of apnea continued.
9 min	Respiration improved, but animal remained completely comatose.
11 min	The respiratory rate improved; however, the animal remained completely unconscious and unresponsive to touch.
12 min	The animal made periodic head movements but remained unconscious: respiration was nearly normal.
18 min	There was no significant change; the animal remained completely unresponsive and unconscious.
22 min	Some visual response was evident as the animal responded to the light of a flashlight.
36 min	The animal remained unconscious and was returned to the cage.

50 min	Respiration was regular, but the animal remained unconscious, lying on the floor of the cage and making no attempt to get up.
1 hr, 15 min	The animal was responsive to touch but made no effort to get up.
2 hr	The animal was able to stand and to remain standing by holding onto the sides of the cage. The animal made some unsuccessful attempts to eat an orange.
4 hr	The animal remained sitting on the floor of the cage with some tremors of the upper and lower limbs, but without convulsions or loss of consciousness.
8 hr	The animal appeared normal.
12 hr	The animal was moving about the cage without difficulty and appeared normal.
24 hr	The animal appeared to have recovered completely and did not show signs of relapse throughout the remainder of observation period.

Summary: The intramuscular administration of 100 ug/kg diazepam was sufficient to prevent the onset of severe tonic-clonic convulsions.

MONKEY #9

Time	Event
-4 hr, 30 min	Monkey #13833, weighing 3.2 kg, was given the last oral dose of mestinon equivalent to 1.2 mg/kg of pyridostigmine.
- 20 min	A 2 ml blood sample was withdrawn for cholinesterase activity determination. Red cell cholinesterase activity was inhibited 35%.
0 min	5 LD50 (76.5 ug/kg) of soman was injected intramuscularly into the gastrocnemius muscle mass of the left hind limb.
1 min	Atropine, 2-PAM and 100 ug/kg of diazepam were injected intramuscularly into the quadriceps muscle mass of the right hind limb.
2 min	The animal attempted to stay on its feet exhibiting some incoordination and jerking movements.
3 min	The animal lay quietly on its side exhibiting periods of prolonged apnea.
4 min	The animal was very quiet, demonstrating no movement or response to touch. There was no indication of an onset of convulsions.
5 min	The respiration had improved to a deeper, faster pattern.
7 min	Some excess salivation and some respiratory obstruction were evident. The eyelids were moving in a rhythmic fluttering pattern, but there was no peripheral indication of tonic-clonic convulsions.
9 min	The animal was quiet and unresponsive to touch.
15 min	The animal continued to be comatose, unresponsive to touch or the light of a flashlight. Respiration was rapid, regular and clear.
25 min	The animal continued to be unconscious, demonstrating no signs of convulsions.
42 min	Animal continued in a comatose state; the respiration was slightly obstructed.
1 hr, 30 min	The animal was returned to the cage where it continued to lie on its side in a semiconscious state.

4 hr	The animal had recovered consciousness and appeared normal except for some excessive salivation and mild tremors and fasciculations of the muscles of the back and hind limbs.
8 hr	The animal continued to respond normally.
24 hr	The animal appeared to have recovered completely and did not regress or relapse during the 48-hr observation period.

Summary: The dose of 100 ug/kg of diazepam prevented the onset of severe tonic-clonic convulsions.

MONKEY #10

TIME	EVENT
-4 hr 30 min	Monkey #13842, weighing 3.3 kg, was given the last oral dose of mestinon equivalent to 1.2mg/kg of pyridostigmine.
-20 min	2 ml of blood were withdrawn for cholinesterase activity determination. Red cell acetylcholinesterase activity was inhibited 36%.
0 min	5 LD50 (76.5 ug/kg) of soman was injected intramuscularly into the gastrocnemius muscle mass of the left hind limb.
1 min	Atropine, 2-PAM and 100 ug/kg of diazepam were injected intramuscularly into the quadriceps muscle mass of the right hind limb.
2 min	The animal lay on its side unconscious; respiration stopped abruptly.
3 min	The animal continued to lay on its side completely unconscious with no signs of respiration.
4 min	The animal produced its first deep respiratory gasp. There was no movement and no signs of consciousness.
5 min	The animal produced several deep respiratory gasps with momentary apnea. The animal remained unconscious and demonstrated no response to touch.
7 min	The periods of apnea, followed by deep respiratory gasps continued. The animal remained completely unconscious.
9 min	Respiration was more regular. There was no response to touch and no movement of the limbs, but some periodic movement of the head.
11 min	Respiration was regular and adequate with some irregular twitching movements of the limbs.
13 min	Movement of the fore limbs and hind limbs had increased. There were no indications of convulsions, no response to touch or any indication of consciousness.
16 min	The movement in the hind limbs progressed then stopped and were followed by periods of quiescence.

18 min	The movement of the hind limbs continued, there were no signs of movement in the fore limbs, no response to touch or light.
22 min	There was a periodic tremor in the hind limbs which stopped for short intervals.
25 min	Severe hind limb tremors occurred which would stop periodically. There was no involvement of the fore limbs or head and neck. The animal remained completely unconscious.
28 min	The animal had some severe tremors of the hind limbs followed by a period of quiescence.
33 min	Tonic-clonic convulsions occurred which were definite and severe, but they only occurred for a period of 30 seconds and then the animal was completely quiet.
36 min	A brief period (15 seconds) of severe convulsions occurred and then the animal was quiet.
37 min	Another 15-second interval of severe convulsions occurred followed by a period of quiescence.
44 min	The animal remained quiet since the 37-minute observation. No additional convulsive movements were observed.
53 min	The animal remained completely quiescent demonstrating no response to touch or light.
1 hr 5 min	The animal was returned to its holding cage.
1 hr 30 min	The animal was able to raise its head slightly. The animal appeared semiconscious but made no attempt to get up.
2 hr	The animal continued lying on the floor of the cage, demonstrated some tremors, and fasciculations along the neck and shoulders of the animal.
4 hr	The animal was able to sit up and move around in the cage.
6 hr	The animal was able to move about in the cage, but appeared weak, disoriented, and fatigued. The tremors and fasciculation of the upper and lower body continued.
8 hr	The animal was able to move about in the cage in an uncoordinated manner.

12 hr	No significant improvement during this last 4 hours.
24 hr	The animal appeared to be normal, was able to move about the cage with ease, and ate without difficulty. The animal remained normal during the remainder of the 48-hr observation period.

Summary: The dose of 100 ug/kg of diazepam did not prevent the onset of several short periods of tonic-clonic convulsions in this animal.

MONKEY #11

Time	Event
-4 hr, 30 min	Monkey #13839, weighing 2.9 kg, was given the last oral dose of mestinon equivalent to 1.2 mg/kg of pyridostigmine.
-20 min	A 2 ml blood sample was drawn for cholinesterase activity determination. Red cell cholinesterase activity was inhibited 37%.
0 min	5 LD50 (76.5 ug/kg) of soman was injected intramuscularly into the gastrocnemius muscle mass of the left hind limb.
1 min	Atropine, 2-PAM and 100 ug/kg diazepam were injected intramuscularly into the quadriceps muscle mass of the right hind limb.
1 min, 30 sec	The animal collapsed and remained lying on its side.
2 min	There was no movement, no respiration or signs of consciousness in the animal.
2.5 min	A respiratory gasp occurred followed by another period of prolonged apnea.
4 min	Another deep respiratory gasp followed by period of apnea.
5 min	Respiration appeared to have recovered; respiration was shallow but regular.
9 min	The animal expired suddenly. Cause of death was assumed to be due to cardiovascular and respiratory collapse.

Distribution List

Addresses	Copies
Defense Technical Information Center ATTN: DTIC-DDAC Cameron Station, Bldg 5 Alexandria, VA 22304-6145	2
Commander US Army Medical Research and Development Command Fort Detrick, MD 21702-5012	2
HQDA(DASG-HCD) Washington, DC 20310	1
Director Walter Reed Army Institute of Research Bldg 40 Washington, DC 20307-5100	1
Commander Letterman Army Institute of Research Bldg 1110 Presidio of San Francisco, CA 94129-6800	1
Commander US Army Aeromedical Research Laboratory ATTN: Scientific Information Center P.O. Box 577 Fort Rucker, AL 36362-5000	1
Commander US Army Biomedical Research and Development Laboratory Bldg 568 Fort Detrick, MD 21702-5010	1
US Army Medical Research Institute of Infectious Disease Bldg 1425 Fort Detrick, MD 21702-5011	
Commander US Army Research Institute of Environmental Medicine Bldg 42 Natick, MA 01760-5007	1
Commandant US Army Chemical School ATTN: ATZN-CM-C Fort McClellan, AL 36205	1
Director Armed Forces Medical Intelligence Center Fort Detrick, MD 21702-5004	1
Commander US Army Institute of Dental Research Bldg 40 Washington, DC 20307-5300	1
Commander US Army Institute of Surgical Research Bldg 2653 Fort Sam Houston, TX 78234-6200	1
Commandant Academy of Health Sciences US Army ATTN: HSHA-CDC Fort Sam Houston, TX 78234-6100	1
Commandant Academy of Health Sciences US Army ATTN: HSHA-CDM Fort Sam Houston, TX 782346100	1
Dr. Joseph Osterman Director, Environmental and Life Sciences Office of the Deputy Under Secretary of Defense (Rsch & Adv Technology) Room 3D129 Washington, DC 20301-2300	1
Commander US Army Training and Doctrine Command ATTN: ATMD Fort Monroe, VA 23651	1

Commander 1
US Army Nuclear and Chemical Agency
7500 Backlick Road
Bldg 2073
Springfield, VA 22150-3198

Biological Science Division 1
Office of Naval Research
Arlington, VA 22217

Executive Officer 1
Naval Medical Research Institute
Naval Medicine Command
National Capital Region
Bethesda, MD 20814

USAF Armstrong Laboratory/CFTO 1
Sustained Operations Branch
Brooks AFB, TX 78235-5000

Department of Health and Human Services 1
National Institutes of Health
The National Library of Medicine
Serial Records Section
8600 Rockville Pike
Bethesda, MD 20894

Stemson Library 1
Academy of Health Sciences
Bldg 2840, Rm 106
Fort Sam Houston, TX 78234-6100

US Army Research Office 1
ATTN: Chemical and Biological
Sciences Division
P.O. Box 12211
Research Triangle Park, NC 27709-2211

AFOSR/NL 1
Bldg 410, Rm A217
Bolling AFB, DC 20332

Commander 1
US Army Chemical Research,
Development & Engineering Center
ATTN: SMCCR-MIS
Aberdeen Proving Ground, MD 21010-5423

LTC Don W. Korte, Jr. 1
Battelle Memorial Institute, JM-3
505 King Avenue
Columbus, OH 43201-2695

Commander 26
US Army Medical Research
Institute of Chemical Defense
ATTN: SGRD-UV-ZA
SGRD-UV-ZB
SGRD-UV-ZS
SGRD-UV-RC (5 copies)
SGRD-UV-R (13 copies)
SGRD-UV-AI-W
SGRD-UV-D
SGRD-UV-P
SGRD-UV-V
SGRD-UV-Y
Aberdeen Proving Ground, MD 21010-5425